

AMENDMENT TO THE SPECIFICATION

Delete paragraph 0002, and add, as follow:

B1 [0002] Japanese Patent ~~Opening~~ Opening Heisei 5-317092 (Gazette), for example, discloses a method wherein a test is performed to discover whether or not an endophyte is living in plant tissue, e.g., callus derived from perennial rye grass, and after introducing an endophyte into callus which is confirmed not to contain endophyte, the endophyte is introduced to perennial rye grass wherein the plant tissue is reproduced.

Delete paragraph 0014, and add, as follows:

B2 [0014] According to one aspect of this invention, there is provided a symbiotic fungus comprising a filamentous fungus whose final metabolic product is chanoclavine. The symbiotic fungus meant here may belong to the genus Neotyphodium ~~Neotyphodium~~. The symbiotic fungus may also be one, two or more types of fungi deposited at the Japanese National Institute of Bioscience and Human Technology under the numbers FERM P-17672, FERM P-17673 and FERM P-17674.

Delete paragraph 0016, and add, as follows:

B3 [0016] The invention relating to a plant, concerns a plant into which the symbiotic fungus whose final metabolic product is chanoclavine, is introduced. Here, the symbiotic fungus may be a filamentous fungus belonging to the genus Neotyphodium ~~Neotyphodium~~. The plant into which the symbiotic fungus is artificially introduced may be a grass which is any of Agrostis, Festuca, Poa and Lolium. Later generations of seeds taken from these plants, plants grown from later generations of seeds, or hybrid plants having these plants and seeds as parents, are also within the scope of this invention.

Delete paragraph 0020, and add, as follows:

B4 [0020] The present Application, which specifically concerns a symbiotic fungus which biosynthesizes chanoclavine as its final metabolic product, involves screening before and after introduction of the fungus into the plant, followed by infection of the plant with the fungus. Filamentous fungi belonging to the genus Neotyphodium

B4 Neotyphodium are examples of symbiotic fungi which infect grasses.

Delete paragraphs 0052 and 0053, and add, as follows:

B5 [0052] To isolate the endophyte from the plant, the leaf and leaf sheath were washed with water, immersed in a 70% aqueous solution of ethanol for 10 seconds, immersed in a 2.5% aqueous solution of sodium hypochlorite for 10 minutes, washed three times with sterile water, transferred to an endophyte isolation culture, and cultured in the dark at 25[±]e °C.

[0053] The isolation culture was prepared by sterilizing PDA (potato dextrose agar) adjusted to pH 5.6 at 121 [±]e °C for 15 minutes, adding 100 mg/l each of penicillin and streptomycin, and pipetting 20 ml portions into plastic Petri dishes of diameter 9 cm.

Delete paragraph 0057, and add, as follows:

B6 [0057] A PDA culture of thickness 2-3 mm was mounted on a glass slide, mycelium was grown on the culture, and the morphology of the mycelium and formation of conidiospores was examined. This culture was performed at 25[±]e °C in the dark.

Delete paragraph 0061, and add, as follows:

B7 [0061] After 8 days in the dark at 25[±]e °C and 30[±]e °C, the plants were placed under illumination at 15[±]e °C for 16 hours for 4 days, and placed under illumination at 25[±]e °C for 16 hours for at least 2 days. Plants which had turned green were acclimatized in pots.

Delete paragraph 0063, and add, as follows:

B8 [0063] As a result, the endophyte was detected in plants of the genres Agrostis, Festuca, Poa and Lolium, which are grasses. From the life cycle of the fungus, this endophyte was found to be a Neotyphodium ~~Neotyphodium~~ endophyte which reproduces only asexually and does not leave the plant.

Delete paragraph 0066, and add, as follows:

B9 [0066] Seedlings obtained immediately after germination on an MS culture were transplanted to the callus induction cultures, and

B9 cultured for 2 months in the dark at 25[±] °C so as to obtain callus which had differentiating ability. All calluses were induced on the aforesaid induction culture, and then transferred to the MS base culture without addition of plant hormone.

Delete paragraph 0068, and add, as follows:

B10 [0068] The callus was cultured for several weeks in the dark at 25[±] °C and 30[±] °C, then placed under illumination for 16 hours, or alternatively it was placed under illumination for 16 hours from the start. The regenerated plant was then transferred to a fresh MS culture and grown for one month. When an examination was made for presence of the endophyte according to the method described in (1), it was confirmed that the endophyte had been introduced.

(6) Method of screening for fungus which specifically synthesizes chanoclavine in the plant

Delete paragraphs 0072-0074, and add, as follows:

[0072] From the above results, it is clear that chanoclavine is biosynthesized and accumulates in plants infected specifically with the symbiotic fungi which were deposited at the Japanese National Institute of Bioscience and Human Technology, including Neotyphodium Neotyphodium sp. FERM P-17672. Also, it was confirmed that the fungi infecting the plants could be screened for using the biosynthesis and accumulation of chanoclavine as a marker.

B11 [0073] For plants which had been cultivated from later generations of seeds of plants infected with the fungi deposited at the Japanese National Institute of Bioscience and Human Technology, including Neotyphodium Neotyphodium sp. FERM P-17672, the presence or absence of chanoclavine was also confirmed by TLC analysis. It was found that even in later generations of plants, biosynthesis of chanoclavine proceeded as in the case of the original plants.

(7) Method of screening for fungi which specifically synthesize chanoclavine on artificial culture

[0074] Endophytes, e.g., Neotyphodium Neotyphodium sp. FERM

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P-17672, were isolated from plants synthesizing chanoclavine in endophyte-infected plants. To isolate the endophyte, the leaf and leaf sheath were washed with water, immersed in a 70% aqueous solution of ethanol for 10 seconds, immersed in a 2.5% aqueous solution of sodium hypochlorite for 10 minutes, washed three times with sterile water, cut to a size of 5x5 mm, transferred to an endophyte isolation culture, and cultured in the dark at 25[±]0 °C.

Delete paragraphs 0088 and 0089, and add, as follows:

[0088] A test of resistance to webworm which is a major pest in pasture grass was performed using plants belonging to the genus *Poa* infected with, for example, *Neotyphodium* *Neotyphodium* sp. FERM P-17672 by the above method, and plants infected with fungus strains other than the above which were deposited at the Japanese National Institute of Bioscience and Human Technology.

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[0089] Pest resistance tests were performed by releasing approximately 200 webworm larvae immediately after hatching in Petri dishes of diameter 9 cm containing respectively the above leaf sections, leaving in a room at 25[±]0 °C, and examining the extent of damage after 24 hours and again after 48 hours. After 48 hours, whereas almost all the plants infected with fungi other than the fungi deposited at the Japanese National Institute of Bioscience and Human Technology had been consumed, the plants infected with *Neotyphodium* *Neotyphodium* sp. FERM P-176723 had practically all their leaves remaining and showed strong resistance. The results obtained for *Poa* and *Lolium* are respectively shown in Fig. 11 and Fig. 12.
